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Agricultural microbiology in Ukraine: achievements, problems, prospects

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The purpose. To systematize achievements of microbiologists of Ukraine who carry out researches in agrarian science, and to analyze problems and prospects the science. **Methods.** Analysis, synthesis, generalization. **Results.** Basic achievements of Ukrainian microbiologists are resulted in the following spheres: creation of modern microbial specimens for optimization of root nutrition of cultivated plants; determination of physiologically expedient doses of fertilizers in techniques of growing crops at observance of criteria of biological diagnostics; increase of coefficients of assimilation by plants of acting substance from fertilizers at use of biological products; protection of plants against pathogenic funguses, bacteria, viruses and insects; optimization of processes of haying, silage making, conservation of wet flaked grains of corn. Problems demanding solution are contoured. **Conclusions.** Development of microbiologists have great value for implementation of potential of agrarian production and martempering ecological situation in agrocenosis, which need observance of principles of the integrated system of protection of plants, with expansion of use of biomethod. Creation of seed grain improved from viruses is of vital importance for implementation of potential of productivity of agrocenosis, as well as application of probiotics created by Ukrainian microbiologists in processes of preparation of feedstuffs. All that provides growth of quality, inactivation of mycotoxins, warns aerobic spoilage of the tinned feedstuffs.

Key words: *agricultural microbiology, microbial specimens, physiologically expedient doses of fertilizers, protection of plants against pathogenic organisms, probiotic specimens, microbiological aspects of processes of conservation of feedstuffs.*

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Since the introduction of the foundations of microbiology in the XIX century several separate sections have been distinguished, among which agricultural microbiology occupies a special place with the problems, studied by microbiologists, being relevant to the global agrarian production. The most important studies, in the opinion of the author, are laying nowadays in the areas of correction of the microorganisms' composition in the root zone of cultured plants, microbiological diagnostics of the conditions of soil agrocenoses and the substantiation of environmentally appropriate fertilizer systems of agricultural crops, optimization of the processes of degradation and synthesis of humus. In addition, the question of biological of plants protection against diseases and pests as well as the optimization of the development of specific microorganisms at forage conservation are particularly important.

A considerable part of the above-mentioned problems is solved by microbiologists working at the institutions of the National Academy of Agrarian Sciences of Ukraine, conducting studies on the tasks of the SRP07 "Agricultural Microbiology" ("Scientific principles of optimization of microbiological processes in the effective functioning of agroecosystems"), as well as on specific tasks included in other scientific programs.

Within the article, author outlines most important, on his view, economic, environmental and social aspects as it is almost impossible to highlight all the achievements and problems of agricultural microbiology within the single review.

Soil microbiology. Classical works of V. V. Dokuchaiev and P. A. Kostychev show that the formation of a fertile soil layer is a complex process – both geological and biological. P. A. Kostychev, in addition, showed the importance of soil microorganisms in the formation of biologically active soils, demonstrating that microorganisms not only decompose organic residues, but also continuously synthesize complex organic compounds, including biologically active substances that promote the active development of plants. The neglect of importance of natural processes and global use of chemical products in agrarian production have led to degradation of soils and the build-up of a number of environmental problems, including those associated with the status of microorganism groups.

As is known, the plant roots are surrounded with soil microorganisms, served as the trophic intermediaries between the soil and plant [1]. It is microorganisms that convert inorganic soil compounds inaccessible to plants to the ones that can be involved in plants metabolism. According to M. O. Krasylnykov, the role of organisms inhabiting the rhizosphere, resembles the functions of the digestive system of animals. Thus, in the system "soil – microorganisms – plant", bacteria and micromycetes are an indispensable and integral part. However, today, in most agrocenoses soils the number of the certain microorganisms' types which have always been considered as the indicators of soil fertility have been minimized. Their place was taken by bacteria atypical for the soil-forming process. At the same time, individual agrocenoses have become a reservoir of pathogens of plant diseases. In this connection, there is a need in agrarian techniques that can promote the increase of the number of aboriginal agronomically valuable microorganisms, or ensure artificial introduction of the necessary bacteria to agrocenoses. Such approach is justified for practically all modern agrocenoses, as the soils, as the acreage of degraded, in the biological sense, soils is increasing dramatically. That is why in economically developed countries, despite their significant industrial potential, which allows the production and application of fertilizers, especially nitrogen (given the inexhaustibility of raw materials for their production), in large numbers, are now interested in microbiological means for intensification of agricultural production. This is due to both economic considerations and environmental requirements.

Ukrainian microbiologists have created a number of microbial preparations based on active strains of nitrogen fixing, phosphate mobilizing and growth stimulating microorganisms, namely: Albobacteryn, Biogran, Diazobacteryn, Microhumin, Polymyxobacteryn, Rhizohimun, Khetomik (Institute of Agricultural Microbiology and Agroindustrial Manufacture of the NAAS), Biopolitsyd, Rhizoactiv, Rhizobofit (Institute of Agroecology and Environmental Management, NAAS), Azogran (D. K. Zabolotny Institute of Microbiology and Virology, NAS). Ukraine), and others. Developed biopreparations are characterized by high efficiency. Most of them are certified for use in organic farming technology.

New principles for creation of microbial preparations are substantiated, and consider not only the presence of an active bacterial strain in preparation but also the optimal amounts of phytohormones of auxin and cytokinin nature [2-4]. The interaction of the bacterial component with physiologically active compounds provides the formation of valuable plant-bacterial symbiosis and associations and positively affects the yield of agricultural crops and product quality.

The use of biopreparations significantly influences the formation of the plants root system, its absorption capacity, the activity of several plant enzyme systems, that overall helps to optimize the nutrient uptake by plants. According to experiments with a heavy isotope of ^{15}N in studies with lysimeter conducted at the Institute of Agricultural Microbiology and Agroindustrial Manufacture of the National Academy of Sciences, the degree of nitrogen utilization from fertilizers under the application of microbial preparations has increased by 20-30 %, followed by the decrease in the intensity of nutrient compounds migration through the soil profile. In general, according to the results of field experiments and production trials, the effect of biopreparations on the yield of agricultural crops is equivalent to the effect of 30-60 kg/ha of mineral nitrogen and 20-40 kg/ha of phosphorus [5, 6]. The economic efficiency of microbial preparations (under the mean parameters of mineral fertilizer savings) averages 2,800 UAH/ha [4].

It has been established that useful soil microorganisms introduced into the agrocenoses, occupy root zone and block for a long time the infection of plants with pathogenic bacteria and micromycetes. It has been shown that even under the epiphytotic disease development the pre-sowing inoculation of seeds with specific preparations has delayed the development of diseases for 2-3 weeks, which significantly influenced the crops yields. It has been proved that seeds obtained from bacterized plants are less infected by pathogens, especially fungi, which greatly increases preservation of the grain.

The next extremely important issue that solves modern soil microbiology is the establishment of physiologically optimal fertilizer, primarily nitrogen, norms. It can be stated that despite the widest use of biological preparations in the future, agriculture cannot completely abandon the use of mineral fertilizers. But the amount of solid fertilizers must be justified not only for economic reasons, but also for the ecological and physiological expediency of their application, since the excess of nitrogen fertilizers pollute the environment

and lead to the deterioration of physical, chemical and biological properties of soils.

There are different ways to determine the physiologically acceptable doses of fertilizers. However, the most reliable parameters of the permissible limits in agrochemicals load on agrocenoses are soil microorganisms. Institute of Agricultural Microbiology and Agroindustrial Manufacture of the NAAS has proposed original methodology for the determination of physiologically (ecologically) rational norms of nitrogen fertilizers for agricultural crops, based on the comparison of the parameters of the course of two opposite processes in the nitrogen cycle – nitrogen fixation and biological denitrification [6, 7]. Phosphorus and potassium fertilizers are proposed to be applied in balance to the defined norms of mineral nitrogen. The gas chromatographic determination of nitrogen fixing and denitrification activity is extremely sensitive (the accuracy of the determination is approaching 10^{-9} mole of N_2), thus allowing to consider the developed method as the most accurate among the existing ones. Given method was tested on winter wheat, winter rye, spring barley, corn, potatoes and other crops cultivated on different fertilization backgrounds. The highest effectiveness of microbial preparations was determined under the use of ecologically appropriate fertilization systems [8].

The results of the above-mentioned solutions provide the basis for a new strategy of chemical fertilizers application in agricultural production – both within the limits of the physiological optimum and in combination with biological preparations.

The studies conducted in the Institute of Agricultural Microbiology and Agroindustrial Manufacture of the National Academy of Agrarian Sciences of Ukraine have established that the excess amount of mineral nitrogen compounds in soil and lack of raw organic matter promote the development of specific microorganisms and initiates the humus breakdown. It has been shown that in order to avoid this phenomenon, there should be enough raw organic matter in the soil (either from green manure biomass or crop straw) to ensure the temporary binding of excess mineral nitrogen by microorganisms and its transformation into the organic compounds.

Institutions of the National Academy of Agrarian Sciences of Ukraine are actively study means of the efficient composting of organic matter of various origin. Institute of Agricultural Microbiology and Agroindustrial Manufacture NAAS have developed the principles of controlled composting of organic matter (manure and poultry litter), that focus on the introduction of cellulose and lignin destructing microorganisms into the substrate [9, 10]. The Institute also have developed new bioactive organic mineral fertilizers Phosphohumin and Biokom-T [11, 12], that are characterized not only by the beneficial agrochemical composition, but also by high content of agronomically useful microorganisms and phytohormones, thus promoting crop yields and quality. National Scientific Centre “Institute of Soil Science and Agrochemistry named after. O. N. Sokolovskyi” of the NAAS has developed a technology for the production of compost with high content of humus compounds. Organic and mineral bioactive fertilizer Ecobiom was developed in the National Scientific Centre “Institute of Agriculture of the NAAS”. All named fertilizers are being actively introduced into the agricultural production of Ukraine.

At the same time, there are sewage and municipal waste sediments which composting problem has not been fully resolved yet. Even so the ways of obtaining valuable organic matter from municipal waste are known, the microbiological aspects of composting require the development of modern and effective solutions.

Biological plant protection against diseases and pests. Next to the problem of plants root nutrition and the role of soil microorganisms in it is the protection of plants against harmful diseases and pests. This problem is aggravated every year due to the intensive use of chemical plant protection means in agriculture, which leads not only to pollution of the environment, but also to the emergence of new, chemical-resistant forms of pathogens. In this regard, the urgency in the development of biological methods of plant protection, based on the use of natural agents, capable to control the development of harmful species, is beyond doubt.

This issue is currently being actively studied in different countries. Ukrainian scientists have developed several biological preparations to control plant fungal diseases [13, 14], and continue research on such issues as selection of active strains of antagonistic microorganisms and the creation of convenient formulations.

Biofungicides based on living cultures of microorganisms have numerous advantages, like safety to humans and animals, no phytotoxic, mutagenic and carcinogenic activity, as well as a wide range of action in relation to different pathogens. It is also necessary to note their harmlessness to the environment.

The expansion of the spectrum of biological insecticides is also real. Nowadays the most popular biological insecticide, available from our microbiologists, is Bitoxybacyllin and its analogues (based on *Bacillus thuringiensis*) [15]. Overall, the development of bioinsecticides production could improve the situation on the food production market, helping to fight such pests as potato beetle, for example. Extremely promising in this area is the study of antiparasitic effects of metabolites of actinobacteria of the *Streptomyces* genus [16].

The current issue related to the use of biopesticides is control of certain crop diseases, such as black rust, that remain unsolved. In order to address this disease scientists have developed the specific means to disinfect seeding material using electron-beam and microwave processing methods instead of agrochemicals [17]. However, in our opinion, the use of these physical methods should be combined with the subsequent application of biopreparations, including the ones that focus on soil fertility [18]. Such approach seems to be highly efficient for the following reasons: physical treatment may sterilize seeds, as it will kill both pathogenic and beneficial microorganisms. Meanwhile, seeds are evolutionarily adapted to transform some microorganisms' groups required for normal plants functioning to its next generation [19]. Since application of physical methods can break this chain, it is necessary to perform artificial bacterization of sterilized sowing material with beneficial microbiota. In addition, the release of the seed surface from microorganisms leaves an unoccupied ecological niche, which can be taken by pathogenic soil microorganisms.

Another dilemma in crop production is crop protection against viral diseases. Today, the losses from viral infections can reach 30 – 80 %, even so that it can be avoided through the use of clean seeding material. However, besides non-virulent seed production, there are also a number of problems associated with the repeated infection of plants with viruses. Of course, this requires more attention and urgent development of scientific and organizational means to control virus diseases from virologists, which should include monitoring of natural infectious background and the creation of test systems for phytopathogens identification through the formation of phytopathogenic viruses' collection [20]. It is also quite important following the recent discoveries of unidentified viruses in Ukrainian agrocenoses [21].

According to the worldwide experience, the effectiveness of agricultural production to a large extent depends on the identified issues of phytovirological research and practical application of the results.

The question remains whether there is a possibility in future to completely abandon the use of chemical plants protection means against diseases and pests? Probably not, especially considering the diversity of harmful organisms. However, under the optimal functioning conditions of agrocenoses and high farming standards it is possible to reduce the use of agrochemicals or to apply them only if needed. Combined with other methods of plant protection – tillage, scientifically grounded crop rotation, selection of varieties resistant/tolerant to diseases and pests, use of biological preparations can substantially minimize the use of synthetic fungicides and insecticides.

Probiotics and feed microbiology. Diversified man's activities affect the ecological balance and primarily it is associated with the composition of the environmental groups of microorganisms: some microorganisms disappear from ecological niches typical for them while others change their properties (acquire virulence, resistance to antibiotics, change antigenic properties, etc.). The results of such an impact on microorganisms become apparent not only in the change of microbial cenoses of soils, which has already been discussed above, but also in the emergence of new diseases of humans and animals. That said, the influence of anthropogenic factor can hardly be predicted especially for warm-blooded organisms through the food consumption and/or treatment process. And one of the reasons for this is the use of antibiotics.

Elimination of "classic" infections with antibiotics frees ecological niche for new pathogens. In addition, the action of antibiotics in an infected macroorganism is not limited to inhibition of functioning of pathogens, it simultaneously extends to normal microflora, which may result in dysbacteriosis. In addition, prolonged use of antibiotics promotes the formation of populations of microorganisms resistant to medicinal products. In view of the fact that certain diseases of humans and animals are caused by same pathogens, the problem comes to a deadlock. However, this situation can be resolved through the use of bacterial preparations

based on living microbial cultures – probiotics. Their therapeutic and preventive effect is determined by their antagonistic activity to pathogenic and opportunistic microorganisms, the ability to activate macrophages and induction of interferons, as well as their positive effect on the antioxidant system of warm-blooded organisms [22, 23]. The use of probiotics with a therapeutic and preventive purpose, in contrast to antibiotics, stimulates the immune response of the animal body, restores normal microbial cenoses with remaining high quality of livestock products. Probiotics do not have contraindications for use and in combination with veterinary and sanitary measures can have a positive effect not only on the microbial coenosis of gastrointestinal tract, but also on livestock facilities, which has an important epizootic significance.

The promising use of probiotics in the livestock industry is beyond doubt. The D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine and the Institute of Agricultural Microbiology and Agroindustrial Manufacture of the NAAS have developed a wide range of probiotics, which safeness and high enzymatic and antagonistic activity was confirmed in number of performance tests [24]. Considering that the effect is achieved with the small doses and low cost, the use of probiotics in the production becomes an extremely beneficial mean from an economic point of view as well.

The use of probiotics in feed production is also very promising. They are often used for better hay storage under the high humidity conditions and during haylage preparation. The use of probiotics for silage contributes to the preservation and improvement of the feed quality: increases the content of lactic and acetic acids, amylase, reductive sugars, vitamins, suppresses the development of putrid and butyric bacteria. At the same time, silage acquires distinct probiotic properties – it promotes the birth of a healthy offspring, reduces gastrointestinal morbidity of young animals and improves animal productivity [25]. The use of probiotics in the preservation of wet rolled grain is also very promising [26].

Another problem that is hard to solve without the use of probiotics is the silage quality, especially in silage-based feeds of the highest quality that perish quickly (30% in 15 days) under the aerobic conditions (as soon as burrs or silos tranches are opened). Oxygen access to the silage leads to the rapid development of yeast, some bacteria and micromycetes, accumulation of mycotoxins, breakdown of lactic acid and carbohydrates, decomposition of proteins. Applyication of chemical preservatives can correct the situation while there is a better way – to using probiotics during ensiling process. In this case, in addition to the feed preservation, it also indirectly prevents specific diseases of farm animals [27].

Research findings of the scientists working in the field of agricultural microbiology, have been published in recent years in monographs “Experimental soil microbiology”, “Microbiological aspects of optimization of nitrogen fertilization of agricultural crops”, “Microbial preparations in agriculture. Theory and Practice”, “Methodology and practice of using microbial preparations in crop production technologies”, “Biological transformation of nitrogen”, “Soil fungi in plant functioning”, and a number of scientific and practical recommendations. The “Atlas of viruses”, “Catalogue of microorganisms of the useful soil microorganisms’ collection », «Catalogue of scientific developments of the Institute of Agricultural Microbiology of the NAAS» have also been published. Overall, farmers and agrarian producers have been offered a significant amount of scientific and practical recommendations.

Conclusion

Bacteria and microscopic fungi actively influence the production process of cultivated plants, serving as intermediaries between soil and plants to provide them with nutrients. However, due to the unsatisfactory current state of agrocenoses, characterized with the discussed above negative trends, the composition of beneficial microbial groups in soil has been substantially reduced. Under these conditions, the coefficients of nutrients uptake from mineral fertilizers by plants are dramatically low. Observance of the physiologically appropriate fertilizer norms in crop cultivation technologies based on the use of biological indications, as well as the use of modern microbial preparations, developed at the institutions of the National Academy of Agrarian Sciences of Ukraine is seen as an effective solution to the stated problems.

Intensive use of chemical plant protection products leads to the emergence of new forms of pathogens resistant to chemical agents. This requires compliance with the principles of an integrated plant protection system, with the expansion of the use of biological methods. Obtaining virus free seed material is also important for realization of the productivity potential of agrocenoses.

Current animal husbandry technologies should include the use of probiotics. The use the probiotics created by the Ukrainian microbiologists is at no doubt the important stage in feed production (haylage, ensilage and rolled corn grain conservation), ensuring receipt of high product quality, inactivation of mycotoxins, and prevents aerobic spoilage of preserved feed.

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